09/862,004

```
FILE 'EMBASE' ENTERED AT 13:05:02 ON 07 NOV 2003
COPYRIGHT (C) 2003 Elsevier Inc. All rights reserved.

=> s fluorescen## energy transfer# (10a) (multiple or plurality)(10a) wavelength
L1            0 FLUORESCEN## ENERGY TRANSFER# (10A) (MULTIPLE OR PLURALITY)(10A)
                WAVELENGTH

=> s fluorescen## energy transfer# (10a) (differen## or ditinguish####) wavelength#
MISSING OPERATOR UISH####) WAVELENGTH#
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=>  s fluorescen## energy transfer# (10a) (differen## or ditinguish####) (10a)
wavelength#
L2            0 FLUORESCEN## ENERGY TRANSFER# (10A) (DIFFEREN## OR DITINGUISH###
                #) (10A) WAVELENGTH#

=> s  s fluorescen## energy transfer# (10a) (differen## or ditinguish####) (10a)
label#
L3            0 S FLUORESCEN## ENERGY TRANSFER# (10A) (DIFFEREN## OR DITINGUISH#
                ###) (10A) LABEL#

=> s fluorescent energy transfer#(10a)label#
L4           11 FLUORESCENT ENERGY TRANSFER#(10A) LABEL#

=> s l4 and (differen## or distingus####)(10a)wavelength##
L5            0 L4 AND (DIFFEREN## OR DISTINGUS####)(10A) WAVELENGTH##

=> s l4 and wavelenght##
L6            0 L4 AND WAVELENGHT##

=> s l4 and wavelength##
L7            2 L4 AND WAVELENGTH##

=> s l7 and (differen## or ditin######)
L8            1 L7 AND (DIFFEREN## OR DITIN######)

=> s l8 and (multiple or plurality or mitiple)
L9            0 L8 AND (MULTIPLE OR PLURALITY OR MITIPLE)

=> d l8 bib ab kwic

L8   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2003 ACS on STN
AN   1998:509312  CAPLUS
DN   129:132202
TI   Sets of primers labeled with energy transfer fluorescent groups and their
     use in multi-component analysis
IN   Ju, Jingyue
PA   Incyte Pharmaceuticals, Inc., USA
SO   PCT Int. Appl., 31 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.         KIND  DATE           APPLICATION NO.  DATE
     ---------------    ----  --------       ---------------  --------
PI   WO 9831834          A1   19980723       WO 1997-US22914  19971212
         W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
             DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG,
             KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX,
             NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
             UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI,
             FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM,
```

GA, GN, ML, MR, NE, SN, TD, TG

	US 5804386	A	19980908	US 1997-784162	19970115
	US 5814454	A	19980929	US 1997-968327	19971112
	AU 9856022	A1	19980807	AU 1998-56022	19971212
	EP 943019	A1	19990922	EP 1997-952413	19971212
	EP 943019	B1	20030409		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2001509271	T2	20010710	JP 1998-534358	19971212
	AT 236996	E	20030415	AT 1997-952413	19971212
	US 5952180	A	19990914	US 1998-80940	19980519
PRAI	US 1997-784162	A	19970115		
	US 1997-968327	A1	19971112		
	WO 1997-US22914	W	19971212		

AB Sets of **fluorescent energy transfer labels** and methods for their use in multi component anal., particularly nucleic acid enzymic sequencing, are provided. In the subject sets, at least two of the labels are energy transfer labels comprising a common donor and acceptor fluorophore in energy transfer relationship sepd. by **different** distances and capable of providing distinguishable fluorescence emission patterns upon excitation at a common **wavelength**. The subject labels find particular use in a variety of multi component anal. applications, such as probes in FISH and multi array analyses, as well as primers in nucleic acid enzymic sequencing applications.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB Sets of **fluorescent energy transfer labels** and methods for their use in multi component anal., particularly nucleic acid enzymic sequencing, are provided. In the subject sets, at least two of the labels are energy transfer labels comprising a common donor and acceptor fluorophore in energy transfer relationship sepd. by **different** distances and capable of providing distinguishable fluorescence emission patterns upon excitation at a common **wavelength**. The subject labels find particular use in a variety of multi component anal. applications, such as probes in FISH and multi array analyses, as well as primers in nucleic acid enzymic sequencing applications.

=>

09/869,004

WEST

Freeform Search

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: L7 and donor and acceptor

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ **Hit List** ◉ **Hit Count** ○ **Side by Side** ○ **Image**

Search | Clear | Help | Logout | Interrupt

Main Menu | Show S Numbers | Edit S Numbers | Preferences | Cases

Search History

DATE: Friday, November 07, 2003 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L8	L7 and donor and acceptor	3	L8
L7	plum.in.	268	L7
L6	L5 and donor and acceptor	1	L6
L5	L4 and stabilit$3	54	L5
L4	gelfand.in.	623	L4
L3	l1 and stabilit$3	1	L3
L2	L1 and fet	1	L2
L1	breslauer.in.	8	L1

END OF SEARCH HISTORY

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ		
L20	l19 and complex$2	5	L20
L19	L18 and (nucleic acid or polynucleotide)	7	L19
L18	L17 and acceptor$1	7	L18
L17	l15 and ((multiple or plurality) near5 donor$1)	7	L17
L16	L15 and (nuleic acid near5 (donor$1 and acceptor$1))	0	L16
L15	l13 and ((differen$2 or distinguish$3) near5 wavelength$2)	209	L15
L14	L13 and (multiple or multiplex or plurality)	623	L14
L13	fluorescen$2 energy transfer	810	L13
L12	fluorescent energy transfer near5 (multiple or plurality)	0	L12
L11	flurescent energy transfer	0	L11
L10	flurescent energe transfer near5 (differen$2 or distinguish$$) near5 wavelength	0	L10
L9	flurescent energy transfer near5 (multiple or plurality)	0	L9
L8	fluorescent energy transfer near5 (multiple or plurality) near5 label$1	0	L8
L7	L6 and (multiple or plurality or multiplex)	6	L7
L6	fluorescent energy transfer near5 wavelength	9	L6
L5	fluorescent energy transfer near5 (multiple or plurality) near5 wavelength	0	L5
L4	fluorescent energy transfer near5 multiple near5 wavelength	0	L4
L3	L1 and wavelength	1	L3
L2	L1 and (differen$2 near5 wavelength)	0	L2
L1	5532129.pn.	2	L1

END OF SEARCH HISTORY

Generate Collection | Print

Search Results - Record(s) 1 through 7 of 7 returned.

1. 6358684. 27 Aug 99; 19 Mar 02. UV excitable fluorescent energy transfer dyes. Lee; Linda G.. 435/6; 435/91.1 435/91.2 436/94 536/22.1 536/23.1 536/24.3 536/24.33 549/381. C12Q001/68 C12P019/34 C07H019/00 C07H021/00 C07H021/02.

2. 6218124. 27 Aug 99; 17 Apr 01. Method for detecting oligonucleotides using UV light source. Lee; Linda G.. 435/6; 435/91.1 435/91.2 536/22.1 536/23.1 536/24.3. C12Q001/68 C12P019/34 C07H019/00 C07H021/00.

3. 6210896. 13 Aug 99; 03 Apr 01. Molecular motors. Chan; Eugene Y.. 435/6; 422/50 422/82.01 436/156 436/94. C12Q001/68 G01N033/00 G01N025/54.

4. 5952180. 19 May 98; 14 Sep 99. Sets of labeled energy transfer fluorescent primers and their use in multi component analysis. Ju; Jingyue. 435/6; 435/91.2. C12Q001/68 C12P019/34.

5. 5814454. 12 Nov 97; 29 Sep 98. Sets of labeled energy transfer fluorescent primers and their use in multi component analysis. Ju; Jingyue. 435/6; 435/91.2. C12Q001/68 C12P019/34.

6. 5804386. 15 Jan 97; 08 Sep 98. Sets of labeled energy transfer fluorescent primers and their use in multi component analysis. Ju; Jingyue. 435/6; 435/91.2. C12Q001/68 C12P019/34.

7. 5254477. 19 Sep 91; 19 Oct 93. Flourescence intramolecular energy transfer conjugate compositions and detection methods. Walt; David R.. 436/172; 435/11 435/14 435/15 435/4 436/164 436/501 436/536 436/537 436/71 436/95. G01N021/64 G01N021/80 C12Q001/00.

Generate Collection | Print

Term	Documents
NUCLEIC	98020
NUCLEICS	16
ACID	1739708
ACIDS	516282
POLYNUCLEOTIDE	33867
POLYNUCLEOTIDES	18549
(18 AND (POLYNUCLEOTIDE OR (NUCLEIC ADJ ACID))).USPT,JPAB,EPAB,DWPI.	7
(L18 AND (NUCLEIC ACID OR POLYNUCLEOTIDE)).USPT,JPAB,EPAB,DWPI.	7

Previous Page Next Page

Generate Collection

L20: Entry 1 of 5 File: USPT Apr 3, 2001

DOCUMENT-IDENTIFIER: US 6210896 B1
TITLE: Molecular motors

Brief Summary Text (4):
Polymers are involved in diverse and essential functions in living systems. The ability to decipher the function of polymers in these systems is integral to the understanding of the role that the polymer plays within a cell. Often the function of a polymer in a living system is determined by analyzing the structure and determining the relation between the structure and the function of the polymer. By determining the primary sequence in a polymer such as a nucleic acid it is possible to generate expression maps, to determine what proteins are expressed, and to understand where mutations occur in a disease state. Because of the wealth of knowledge that may be obtained from sequencing of polymers many methods have been developed to achieve more rapid and more accurate sequencing methods.

Brief Summary Text (10):
The present invention relates to methods and products for linear analysis of polymers. In particular the invention is based on molecular motors and their use for guiding polymer movement during linear analysis. Recently rapid methods for analyzing polymers using linear analysis techniques have been developed. Such methods are described in co-pending PCT patent application No. PCT/US98/03024 and U.S. Ser. No. 09/134,411, the entire contents of which are hereby incorporated by reference. The method for analyzing polymers described in PCT/US98/03024 is based on the ability to examine each unit of a polymer individually. By examining each unit individually the type of unit and the position of the unit on the backbone of the polymer can be identified. This can be accomplished by positioning a unit at a station and examining a change which occurs when that unit is proximate to the station. The change can arise as a result of an interaction that occurs between the unit and the station or a partner and is specific for the particular unit. For instance if the polymer is a nucleic acid molecule and a T is positioned in proximity to a station a change which is specific for a T could occur. If on the other hand, a G is positioned in proximity to a station then a change which is specific for a G could occur. The specific change which occurs, for example, depends on the station used, the type of polymer being studied, and/or the label used. For instance the change may be an electromagnetic signal which arises as a result of the interaction.

Brief Summary Text (15):
The polymer may be any type of polymer of linked units. The type of molecular motor which can be used, however, will depend on the type of polymer. In one embodiment the polymer is a nucleic acid and the molecular motor is a polymerase. In another embodiment the polymer is a peptide and the molecular motor is a myosin.

Brief Summary Text (19):
The molecular motor tethered to the support may be any type of molecular motor. Preferably the molecular motor is a nucleic acid molecular motor or a peptide molecular motor selected from the group consisting of polymerase, helicase, kinesin, dynein, actin, and myosin.

Brief Summary Text (20):
According to another aspect of the invention a molecular motor is provided. The molecular motor includes an agent positioned in interactive proximity with a signal station of the molecular motor, wherein the agent is selected from the group consisting of an electromagnetic radiation source, a quenching source, and a fluorescence excitation source. In one embodiment the molecular motor is in a solution. In another embodiment, the solution includes only a single molecular motor. Preferably the molecular motor is a nucleic acid molecular motor.

Brief Summary Text (23):
The molecular motor can be a nucleic acid molecular motor or a peptide molecular motor. One type of nucleic acid molecular motor is a polymerase.

Brief Summary Text (30):
The plurality of polymers may be any type of polymer but preferably is a nucleic acid. In one embodiment the plurality of polymers is a homogenous population. In another embodiment the plurality of polymers is a heterogenous population. The polymers can be labeled, randomly or non randomly. Different labels can be used to label different linked units to produce different polymer dependent impulses.

Brief Summary Text (34):
In one embodiment the polymer dependent impulses are optically detectable. In another embodiment the nucleic acids are labeled with an agent selected from the group consisting of an electromagnetic radiation source, a quenching source, a fluorescence excitation source, and a radiation source.

Brief Summary Text (35):
The plurality of polymers may be any type of polymer but preferably is a nucleic acid. In one embodiment the plurality of polymers is a homogenous population. In another embodiment the plurality of polymers is a heterogenous population. The polymers can be labeled, randomly or non randomly. Different labels can be used to label different linked units to produce different polymer dependent impulses.

Brief Summary Text (37):
The plurality of polymers may be any type of polymer but preferably is a nucleic acid. In one embodiment the plurality of polymers is a homogenous population. In another embodiment the plurality of polymers is a heterogenous population. The polymers can be labeled, randomly or non randomly. Different labels can be used to label different linked units to produce different polymer dependent impulses.

Drawing Description Text (8):
SEQ. ID. NO. 1 is a hypothetical nucleic acid sequence.

Drawing Description Text (9):
SEQ. ID. NO. 2 is a hypothetical nucleic acid sequence.

Drawing Description Text (10):
SEQ. ID. NO. 3 is a hypothetical nucleic acid sequence.

Drawing Description Text (11):
SEQ. ID. NO. 4 is a hypothetical nucleic acid sequence.

Detailed Description Text (6):
The unit and the agent are moved relative to one another by a molecular motor. A "molecular motor" as used herein is a biological molecule which physically interacts with a polymer and moves the polymer past a signal station. Preferably the molecular motor is a molecule such as a protein or protein complex that interacts with a polymer and moves with respect to the polymer along the length of the polymer. The molecular motor interacts with each unit of the polymer in a sequential manner. The physical interaction between the molecular motor and the polymer is based on molecular forces occurring between molecules such as, for instance, van der waals forces. The type of molecular motor useful according to the methods of the invention depends on the type of polymer being analyzed. For instance a molecular motor such as e.g., a DNA polymerase or a helicase is useful when the polymer is DNA, a molecular motor such as RNA polymerase is useful when the polymer is RNA, and a molecular motor such as myosin is useful for example when the polymer is a peptide such as actin. Molecular motors include, but are not limited to, helicases, RNA polymerases, DNA polymerases, kinesin, dynein, actin, and myosin. Those of ordinary skill in the art would easily be able to identify other molecular motors useful according to the invention, based on the parameters described herein.

Detailed Description Text (9):
Another preferred type of molecular motor is a helicase. Helicases have previously

been described, e.g., see U.S. Pat. No. 5,888,792. Helicases are proteins which move along nucleic acid backbones and unwind the nucleic acid so that the processes of DNA replication, repair, recombination, transcription, mRNA splicing, translation and ribosomal assembly can take place. Helicases include both RNA and DNA helicases.

Detailed Description Text (12):
The molecular motors of the invention fall into two categories, nucleic acid molecular motors and protein molecular motors. Nucleic acid molecular motors include those molecular motors that move along the backbone of a nucleic acid molecule and include, for instance, polymerases and helicases. The protein molecular motors move along the backbone of a protein or peptide, and include for instance kinesin, dynein, actin and myosin. In some embodiments the molecular motor is preferably a nucleic acid molecular motor and in other embodiments it is preferably a protein molecular motor.

Detailed Description Text (14):
The method of the invention is described with respect to the following non-limiting example, which is provided for illustrative purposes only. The example refers to the analysis of DNA and fluorescence, but those of ordinary skill in the art would understand that it is applicable to all polymers and all claimed detection systems. In the example, a DNA polymerase is labeled with several fluorescent molecules, e.g. donor fluorescent molecules. A DNA molecule labeled with a matching fluorophore, e.g. an acceptor fluorophore, is then used as a template for the DNA polymerase which begins to undergo primer extension. As the acceptor fluorophore moves past the donor fluorophore, fluorescence resonance energy transfer (FRET) occurs. FRET occurs when the donor and acceptor fluorophores undergo a close range interaction in the range of approximately 1 angstrom to 100 angstroms. This distance is achieved when a single nucleotide with a label passes the fluorophore on the polymerase.

Detailed Description Text (15):
FRET analysis using molecular motors can be performed on single molecules in solution or as parallel reactions on a solid planer medium. It may also be performed in parallel reactions in different solutions such as in multi-well dishes. In the embodiment in which the reaction is carried out on a planer solid medium, either the labeled polymer or the labeled molecular motor may be immobilized directly or through a linker onto the surface. If the polymer is attached to the surface, then molecular motor can be added subsequently and if the molecular motor is tethered to the surface, then the polymer may be added to initiate the reaction. In this manner, simultaneous linear reading of multiple donor-acceptor reaction sites can occur to enhance the throughput of the system. When the molecular motor is a DNA polymerase, the sequence of several kilobases of DNA can be obtained rapidly. The approximate rate of sequencing can approach 1 megabase/hour with a 1 camera system.

Detailed Description Text (16):
The preparation of fluorescently labeled enzyme and protein complexes which can serve as molecular motors, is well known in the art. The availability of multiple amine, carboxyl, and sulflfydryl sites on enzymes makes conjugation of labels to these molecules straightforward. Many proteins have been functionalized to produce fluorescent derivatives without loss of activity, including, for instance, antibodies, horseradish peroxidase, glucose oxidase, b-galactosidase, alkaline phosphatase, actin, and myosin. Molecular motors can be easily derivatized in a similar manner, without losing functional activity. Additionally, labels can be incorporated into the polymer using methods known in the art, such as those described in U.S. Ser. No. 09/134,411. For instance, the label can be incorporated into the polymer using commercially available nucleotide or amino acid polymers or as succinimydal ester derivatives which can be linked to primary amino groups.

Detailed Description Text (19):
In a preferred embodiment the fluorescent dye and its energy transfer pair is carefully selected to maximize signal production. This can be accomplished by considering the parameters described by the formula set forth below. Fluorescence energy transfer (FRET) directly related to the spectral overlap of the donor fluorescence emission and the acceptor fluorescence absorbance is determined as J, the normalized spectral overlap of the donor emission (fD) acceptor absorption (.epsilon..sub.A), 90 is the quantum efficiency (or quantum yield) for donor emission in the absence of acceptor (90 is the number of photons emitted divided by number of

photons absorbed), n is the index of refraction (typically 1.3-1.4), and (.kappa.C.sup.2 is a geometric factor related to the relative angle of the two transition dipoles. The equation which summarizes the importance of the normalized spectral overlap is given as:

Detailed Description Text (20):
The J factor is especially important in the determination of the Forster energy transfer distance which is the distance at which energy transfer from donor fluorophore to acceptor fluorophore is 50%. The Forster distance also determines the resolution of the FRET sequencing method. In general the Forster distance can be varied to be between as small as 5 angstroms and 100 angstroms.

Detailed Description Text (21):
We have considered these variables in our choice of the optimal donor-acceptor pair for use in our FRET sequencing system. The J factor is important, but there are additional factors which should be worked into the system for optimal performance such as 1) the sharpness of the spectral bands, 2) the lack of crosstalk between the spectral bands, 3) the ability to immobilize the chosen labels in a polymeric matrix, and 4) the ability to have a match with common labels used for incorporation into DNA.

Detailed Description Text (22):
Other factors can be considered in choosing the proper fluorescent label pair. For instance, the spectral overlap of the labels should be sufficient for energy transfer. By minimalizing direct excitation of the acceptor fluorophore crosstalk in excitation levels can be avoided. Additionally, the emission of the donor fluorophore should not interfere with the detection band from the acceptor fluorophore. In this manner, the measured fluorescent events will be suitable and indicative of the occurrence of energy transfer. Under ideal conditions, the donor and acceptor fluorescence is sharp and not subject to spectral broadening. Furthermore, there are considerations in the quantum yield, photostability, and cross-sectional areas of the labels. All of these parameters can easily be manipulated by one of skill in the art based on the known properties of known and commercially available labels.

Detailed Description Text (23):
Those of ordinary skill in the art can verify the extent of fluorescent labeling of the molecular motor and/or polymer. The level of fluorescence labeling in the fluorophore conjugated molecule is determined by either the absorbance or the fluorescence emission of the sample. The number of fluorophore molecules per molecule is called the F/M ratio. This value is measured for all preparations of enzyme-fluorophore complexes. The ideal F/M ratio is determined for the particular molecule (molecular motor or polymer) molecule-fluorophore combination. Using the known extinction coefficient of the fluorophore, a determination of the derivitization level can be made after excess of the fluorophore is removed.

Detailed Description Text (24):
The activity of the labeled molecular motors can be verified using standard assays which assess the viability of the molecular motor fluorophore complex after conjugation and purification. Various molecular motors have their own assays for activity verification. DNA polymerase and its activity after conjugation to FITC is discussed below to clarify further on this subject. This example is in no way limiting of the scope of the invention.

Detailed Description Text (25):
DNA polymerase-fluorophore complexes are checked in dideoxy sequencing reactions to verify the ability of the modified molecular motor to perform its chain extension function. Primer annealing, labeling, and termination reactions are executed to determine the length of single-stranded, dideoxy terminated products and also to assay the base accuracy of the extended products. The reaction mixtures for the four dideoxynucleotides are subjected to four color automated capillary gel electrophoresis (such as the ABI 3770) for the final analysis. Match of the sequences with the known M13 ssDNA sequencing template confirms the integrity of the polymerase-fluorophore complexes.

Detailed Description Text (26):

FIG. 1 depicts an array of molecular motors (i.e. DNA polymerases) bound to the surface of a glass slide. The polymerases are labeled with donor fluorescent molecules which have emission spectra which partially overlap the excitation spectra of the acceptor molecule. Template acceptor labeled polymer (i.e. DNA) is provided in the reaction mixture along with the appropriate extension primers. The reaction is initiated with a mixture of deoxynucleotides. The chain extension allows the acceptor on the template DNA to be moved in proximity to the donors on the polymerase. Once the acceptor comes within energy transfer proximity to the donor on the immobilized polymerase molecule, non-radiative energy occurs. Sensitized fluorescence emission from the acceptor is induced. The temporally spaced fluorescence emission from the substrates allows for interrogation of the nucleotide information about the template molecule.

Detailed Description Text (28):
Another example of the linear analysis method of the invention is depicted in FIG. 2. In the example the template may be fixed to the glass surface and the polymerase mobile in solution. As shown in FIG. 2, the donor fluorescence molecule may be located on the DNA molecule as opposed to the acceptor. The series of interactions may be mediated by a different molecular motor such as a helicase molecule which unwinds duplex DNA. In this scenario, the helicase molecule is fluorescently tagged and allowed to unwind complexes which are asymmetrically labeled with the fluorescent molecules. The asymmetric labeling allows for the ease of deciphering the information about the polymer.

Detailed Description Text (32):
The methods of the invention also are useful for identifying other structural properties of polymers. The structural information obtained by analyzing a polymer according to the methods of the invention may include the identification of characteristic properties of the polymer which (in turn) allows, for example, for the identification of the presence of a polymer in a sample or a determination of the relatedness of polymers, identification of the size of the polymer, identification of the proximity or distance between two or more individual units of a polymer, identification of the order of two or more individual units within a polymer, and/or identification of the general composition of the units of the polymer. Such characteristics are useful for a variety of purposes such as determining the presence or absence of a particular polymer in a sample. For instance when the polymer is a nucleic acid the methods of the invention may be used to determine whether a particular genetic sequence is expressed in a cell or tissue. The presence or absence of a particular sequence can be established by determining whether any polymers within the sample express a characteristic pattern of individual units which is only found in the polymer of interest i.e., by comparing the detected signals to a known pattern of signals characteristic of a known polymer to determine the relatedness of the polymer being analyzed to the known polymer. The entire sequence of the polymer of interest does not need to be determined in order to establish the presence or absence of the polymer in the sample. Similarly the methods may be useful for comparing the signals detected from one polymer to a pattern of signals from another polymer to determine the relatedness of the two polymers.

Detailed Description Text (38):
As used herein "similar polymers" are polymers which have at least one overlapping region. Similar polymers may be a homogeneous population of polymers or a heterogenous population of polymers. A "homogeneous population" of polymers as used herein is a group of identical polymers. A "heterogenous population" of similar polymers is a group of similar polymers which are not identical but which include at least one overlapping region of identical units. An overlapping region in a nucleic acid typically consists of at least 10 contiguous nucleotides. In some cases an overlapping region consists of at least 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides.

Detailed Description Text (39):
A "polymer" as used herein is a compound having a linear backbone of individual units which are linked together by linkages. In some cases the backbone of the polymer may be branched. Preferably the backbone is unbranched. The term "backbone" is given its usual meaning in the field of polymer chemistry. The polymers may be heterogeneous in backbone composition thereby containing any possible combination of polymer units

linked together such as peptide- nucleic acids (which have amino acids linked to nucleic acids and have enhanced stability). In a preferred embodiment the polymers are homogeneous in backbone composition and are, for example, nucleic acids, polypeptides, polysaccharides, carbohydrates, polyurethanes, polycarbonates, polyureas, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, polyacetates, polyamides, polyesters, or polythioesters. In the most preferred embodiments, the polymer is a nucleic acid or a polypeptide. A "nucleic acid" as used herein is a biopolymer comprised of nucleotides, such as deoxyribose nucleic acid (DNA) or ribose nucleic acid (RNA). A polypeptide as used herein is a biopolymer comprised of linked amino acids.

Detailed Description Text (42):
Whenever a nucleic acid is represented by a sequence of letters it will be understood that the nucleotides are in 5'.fwdarw.3' order from left to right and that "A" denotes adenosine, "C" denotes cytidine, "G" denotes guanosine, "T" denotes thymidine, and "U" denotes uracil unless otherwise noted.

Detailed Description Text (45):
Many naturally occurring units of a polymer are light emitting compounds or quenchers. For instance, nucleotides of native nucleic acid molecules have distinct absorption spectra, e.g., A, G, T, C, and U have absorption maximums at 259 nm, 252 nm, 267 um, 271 nm, and 258 nm respectively. Modified units which include intrinsic labels may also be incorporated into polymers. A nucleic acid molecule may include, for example, any of the following modified nucleotide units which have the characteristic energy emission patterns of a light emitting compound or a quenching compound: 2,4-dithiouracil, 2,4-Diselenouracil, hypoxanthine, mercaptopurine, 2-aminopurine, and selenopurine.

Detailed Description Text (51):
As used herein the "relatedness of polymers" can be determined by identifying a characteristic pattern of a polymer which is unique to that polymer. For instance if the polymer is a nucleic acid then virtually any sequence of 10 contiguous nucleotides within the polymer would be a unique characteristic of that nucleic acid molecule. Any other nucleic acid molecule which displayed an identical sequence of 10 nucleotides would be a related polymer.

Detailed Description Text (67):
The information contained in the data and how it is analyzed depends on the number and type of labeled units that were caused to interact with the agent to generate signals. For instance if every labeled unit of a single polymer, each type of labeled unit (e.g., all the A's of a nucleic acid) having a specific type of label, is labeled then it will be possible to determine from analysis of a single polymer the order of every labeled unit within the polymer. If, however, only one of the four types of units of a nucleic acid is labeled then more data will be required to determine the complete sequence of the nucleic acid. Additionally, the method of data analysis will vary depending on whether the nucleic acid is single stranded or double stranded or otherwise complexed. Several labeling schemes and methods for analysis using the computer system data produced by those schemes are described in more detail below.

Detailed Description Text (69):
A four nucleotide labeling scheme can be created where the A's, C's, G's, and T's of a target DNA is labeled with different labels. Such molecule, if moved linearly past a station, will generate a linear order of signals which correspond to the linear sequence of nucleotides on the target DNA. The advantage of using a four nucleotide strategy is its ease of data interpretation and the fact that the entire sequence of labeled units can be determined from a single labeled nucleic acid. Adding extrinsic labels to all four bases, however, may cause stearic hindrance problems. In order to reduce this problem the intrinsic properties of some or all of the nucleotides may be used to label the nucleotides. As discussed above, nucleotides are intrinsically labeled because each of the purines and pyrimidines have distinct absorption spectra properties. In each of the labeling schemes described herein the nucleotides may be either extrinsically or intrinsically labeled but it is preferred that at least some of the nucleotides are intrinsically labeled when the four nucleotide labeling method is used. It is also preferred that when extrinsic labels are used with the four nucleotide labeling scheme that the labels be small and neutral in charge to reduce

stearic hindrance.

Detailed Description Text (70):
A three nucleotide labeling scheme in which three of the four nucleotides are labeled may also be performed. When only three of the four nucleotides are labeled analysis of the data generated by the methods of the invention is more complicated than when all four nucleotides are labeled. The data is more complicated because the number and position of the nucleotides of the fourth unlabeled type must be determined separately. One method for determining the number and position of the fourth nucleotide utilizes analysis of two different sets of labeled nucleic acid molecules. For instance, one nucleic acid molecule may be labeled with A, C, and G, and another with C, G, and T. Analysis of the linear order of labeled nucleotides from the two sets yields sequence data. The three nucleotides chosen for each set can have many different possibilities as long as the two sets contain all four labeled nucleotides. For example, the set ACG can be paired with a set of labeled CGT, ACT or AGT.

Detailed Description Text (71):
The sequence including the fourth nucleotide also may be determined by using only a single labeled nucleic acid rather then a set of at least two differently labeled nucleic acids using a negative labeling strategy to identify the position of the fourth nucleotide on the nucleic acid. Negative labeling involves the identification of sequence information based on units which are not labeled. For instance, when three of the nucleotides of a nucleic acid molecule are labeled with a label which provides a single type of signal, the points along the nucleic acid backbone which are not labeled must be due to the fourth nucleotide. This can be accomplished by determining the distance between labeled nucleotides on a nucleic acid molecule. For example A, C, and G are labeled and the detectable signals generated indicated that the nucleic acid molecule had a sequence of AGGCAAACG (SEQ. ID. No. 1). If the distances between each of the nucleotides in the nucleic acid molecule are equivalent to the known inter-nucleotide distance for a particular combination of nucleotides except the distance between G and G is twice the normal inter-nucleotide distance then a T is positioned between the two G's and the entire molecule has a sequence of AGTGCAAACG (SEQ. ID. No. 2). The distance between nucleotides can be determined in several ways. Firstly, the nucleic acid and the station may be moved relative to one another in a linear manner and at a constant rate of speed such that a single labeled unit of the nucleic acid molecule will pass the station at a single time interval. If two time intervals elapse between detectable signals then the unlabeled nucleotide which is not capable of producing a detectable signal is present within that position. This method of determining the distance between labeled units is discussed in more detail below in reference to random one nucleotide labeling. Alternatively the nucleic acid and the station may be caused to interact with one another such that each labeled unit interacts simultaneously with a station to produce simultaneous detectable signals. Each detectable signal generated occurs at the point along the nucleic acid where the labeled unit is positioned. The distance between the detectable signals can be calculated directly to determine whether an unlabeled labeled unit is positioned anywhere along the nucleic acid molecule.

Detailed Description Text (72):
Nucleic acid molecules may also be labeled according to a two nucleotide labeling scheme. Six sets of two nucleotide labeled nucleic acid molecule can be used to resolve the data and interpret the nucleotide sequence. Ambrose et al., 1993 and Harding and Keller, 1992 have demonstrated the synthesis of large fluorescent DNA molecules with two of the nucleotides completely extrinsically labeled. The average size of the molecules were 7 kilobases. Six different combinations of two nucleotide labeling are possible using the following formula: ##EQU1##

Detailed Description Text (73):
where n nucleotides are taken k at a time. The possible combinations are AC, AG, AT, CG, CT, and GT. Knowledge of the linear order of the labels in each of the sets allows for successful reconstruction of the nucleic acid sequence. Using a 4-mer (5'ACGT'3) as a model sequence, the theory can be demonstrated. The first set, AC, gives the information that there must be a C after the A. This does not give information about the number of nucleotides intervening the A and the C nor does it give information about any G's or T's preceding the A. The second set, AG, shows that there is also a G after the A. Set AT shows there is a T after the A. From these three sets, it is then

known that the target DNA is a 4-mer and that one C, one G, and one T follow the A. The subsequent sets give information on the ordering of these three nucleotides following the A. Set CG shows that G follows C. Set CT shows that T follows C. Set GT finishes the arrangement to give the final deciphered sequence of 5'ACGT'3 (SEQ ID NO. 4). In addition to the method using six labeled sets of nucleic acid molecules, the sequence can be established by combing information about the distance between labeled nucleotides generating detectable signals as described above and information obtained from fewer than six sets of two nucleotide labeled nucleic acid molecules.

Detailed Description Text (79):
In the population method the entire population of labeled nucleotide is considered. Knowledge of the length of the localized region of the agent and instantaneous rate, as required for the rate method, is not necessary. Use of population analyses statistically eliminates the need for precision measurements on individual nucleic acid molecules.

Detailed Description Text (80):
An example of population analyses using five nucleic acid molecules each traversing a nanochannel is described below. Five molecules representing a population of identical DNA fragments are prepared. In a constant electric field, the time of detection between the first and second labeled nucleotide should be identical for all the DNA molecules. Under experimental conditions, these times differ slightly, leading to a Gaussian distribution of times. The peak of the Gaussian distribution is characteristic of the distance of separation (d) between two labeled nucleotides.

Detailed Description Text (81):
An additional example utilizing a population of one nucleotide randomly labeled nucleic acid molecule (six molecules represent the population) further illustrates the concept of population analysis and the determination of distance information. The nucleic acid is end-labeled to provide a reference point. With enough nucleic acid molecules, the distance between any two A's can be determined. Two molecules, when considered as a sub-population, convey the nucleotide separation molecules, distributions of 4 and 6 nucleotide separations are created. Extending the same logic to rest of the population, the positions of all the A's on the DNA can be determined. The entire sequence is generated by repeating the process for the other three bases (C, G, and T).

Detailed Description Text (82):
In addition to labeling all of one type of labeled unit in the above-described examples, it is possible to use various labeling schemes where not every nucleotide of the nucleotides or markers to be labeled is labeled, such as a one nucleotide labeling scheme where less than all of the one nucleotide are labeled. A representative population of random A-labeled fragments for a 16-mer with the sequence 5'ACGTACGTACGTACGT'3 (SEQ. ID. No. 3) is used. Each individually labeled DNA molecule has half of its A's labeled in addition to 5' and 3' end labels. With a large population of randomly labeled fragments, the distance between every successive A on the target DNA can be found. The end labels serve to identify the distance between the ends of the DNA and the first A. Repeating the same analysis for the other nucleotides generates the sequence of the 16-mer by compiling the data to identify the position of all of the As within that population of nucleic acid molecules. These steps can then be repeated using labeled units for the other nucleotides in the population of nucleic acids. The advantages of using such a method includes lack of steric effects and ease of labeling. This type of labeling is referred to as random labeling. A nucleic acid which is "randomly labeled" is one in which fewer than all of a particular type of labeled unit are labeled. It is unknown which labeled units of a particular type of a randomly labeled nucleic acid are labeled.

Detailed Description Text (83):
A similar type of analysis may be performed by labeling each of the four nucleotides incompletely but simultaneously within a population. For instance, each of the four nucleotides may be partially labeled with its own labeled unit which gives rise to a different physical characteristic, such as color, size, etc. This can be accomplished to generate a data set containing information about all of the nucleotides from a single population analysis. For instance the method may be accomplished by partially labeling two nucleotide pairs at one time. Two nucleotide labeling is possible through

the lowering of steric hindrance effects by using labeled units which recognize the two nucleotides of a nucleic acid strand and which contain a label such as a single fluorescent molecule. Ambrose et al., 1993 and Harding and Keller, 1992 have demonstrated that large fluorescent nucleic acid molecules with two of the nucleotides completely labeled are possible to achieve. The average size of the molecules studied were 7 KB. Partial labeling of two and three nucleotides is possible. For instance, each of three nucleotides is partially labeled with a different labeled unit. In this case, a population of single stranded nucleic acid molecules which are partially labeled with three specific nucleotide pair combinations is generated and can be analyzed.

Detailed Description Text (84):
The methods of the invention can also be achieved using a double stranded nucleic acid. In a double stranded nucleic acid, when a single nucleotide on two of the strands is labeled, information about two nucleotides becomes available for each of the strands. For instance, in the random and partial labeling of A's, knowledge about the A's and T's becomes available. For example, a labeling strategy in which two differently labeled nucleic acid samples can be prepared. The first sample has two non-complimentary nucleotides randomly labeled with the same fluorophore. Non-complimentary pairs of nucleotides are AC, AG, TC, and TG. The second sample has one of its nucleotides randomly labeled. The nucleotide chosen for the second sample may be any one of the four nucleotides. In the example provided, the two non-complimentary nucleotides are chosen to be A and C, and the single nucleotide is chosen to be A. Two samples are prepared, one with labeled A's and C's and another with labeled A's. The nucleic acid is genomically digested, end labeled, purified, and analyzed. Such procedures are well-known to those of ordinary skill in the art. The information from each fragment is sorted into one of two complimentary strand groups. Sorting the information into different groups allows the population analysis to determine the positions of all the desired nucleotides. The first group of data provides known positions of all the A's and C's on one strand. The second group of data provides known positions of all of the A's. The combination of these two data sets reveals the position of all of the A's and C's on one strand. The same procedure may be applied to the complimentary strand to determine the positions of the A's and C's on that strand. The resultant data reveals the entire sequence for both strands of the nucleic acid, based on the assumption that the strand includes the complimentary nucleotide pairs of A and C (A:T and C:G). To cross-verify the sequence, the process can be repeated for the other pairs of non-complimentary nucleotides such as TG, TC and AG.

Detailed Description Text (86):
By including more than one physical characteristic into the label, the simultaneous and overlapping reading of the nucleic acid within the same temporal frame may provide more accurate and rapid information about the positions of the labeled nucleotides than when only a single physical characteristic is included. The sample may be, for instance, labeled with different wavelength fluorophores. Each of the fluorophores can be detected separately to provide distinct readings from the same sample. For instance, the end units of a polymer may be labeled with fluorophores which emit at a first wavelength and a set of internal units may be labeled with a fluorophore which emits at a second wavelength. As the polymer is moved past the signal station both wavelengths can be detected to provide information about both sets of labels.

Detailed Description Text (87):
One use for the methods of the invention is to determine the sequence of units within a polymer. Identifying the sequence of units of a polymer, such as a nucleic acid, is an important step in understanding the function of the polymer and determining the role of the polymer in a physiological environment such as a cell or tissue. The sequencing methods currently in use are slow and cumbersome. The methods of the invention are much quicker and generate significantly more sequence data in a very short period of time.

Detailed Description Text (90):
The interaction station in a preferred embodiment is a region of a molecular motor where a localized agent, such as an acceptor fluorophore, attached to the molecular motor or support can interact with a polymer passing through the molecular motor. The point where the polymer passes the localized region of agent is the interaction

station. As each labeled unit of the polymer passes by the agent a detectable signal is generated. The agent may be localized within the region of the channel in a variety of ways. For instance the agent may be physically attached to the molecular motor, directly or by a linker, at the site where the polymer interacts with the molecular motor. Alternatively, the molecular motor may be attached to a support and the agent may also be attached to the support, as long as the agent is attached to a region of the support by which all units of the polymer will pass. For instance, the agent may be embedded in a material or on the surface of a material that forms the wall of a channel wherein the molecular motor is attached to the wall and moves the polymer through the channel. Alternatively the agent may be a light source which is positioned a distance from the molecular motor or support but which is capable of transporting light directly to a region of the channel through a waveguide. These and other related embodiments of the invention are discussed in more detail below. The movement of the polymer may be assisted by the use of a groove or ring to guide the polymer.

Detailed Description Text (98):
A variation of these types of interaction involves the presence of a third element of the interaction, a proximate compound which is involved in generating the signal. For example, a labeled unit may be labeled with a light emissive compound which is a donor fluorophore and a proximate compound can be an acceptor fluorophore. If the light emissive compound is placed in an excited state and brought proximate to the acceptor fluorophore, then energy transfer will occur between the donor and acceptor, generating a signal which can be detected as a measure of the presence of the labeled unit which is light emissive. The light emissive compound can be placed in the "excited" state by exposing it to light (such as a laser beam) or by exposing it to a fluorescence excitation source.

Detailed Description Text (100):
A set of interactions parallel to those described above can be created wherein, however, the light emissive compound is the proximate compound and the labeled unit is either a quenching source or an acceptor source. In these instances the agent is electromagnetic radiation emitted by the proximate compound, and the signal is generated, characteristic of the interaction between the labeled unit and such radiation, by bringing the labeled unit in interactive proximity with the proximate compound.

Detailed Description Text (101):
The mechanisms by which each of these interactions produces a detectable signal is known in the art. For exemplary purposes the mechanism by which a donor and acceptor fluorophore interact according to the invention to produce a detectable signal including practical limitations which are known to result from this type of interaction and methods of reducing or eliminating such limitations is set forth below.

Detailed Description Text (105):
Analysis of the radiolabeled polymers is identical to other means of generating signals. For example, a sample with radiolabeled A's can be analyzed by the system to determine relative spacing of A's on a sample DNA. The time between detection of radiation signals is characteristic of the polymer analyzed. Analysis of four populations of labeled DNA (A's, C's, G's, T's) can yield the sequence of the nucleic acid analyzed. The sequence of DNA can also be analyzed with a more complex scheme including analysis of a combination of dual labeled DNA and singly labeled DNA. Analysis of a and C labeled fragment followed by analysis of a labeled version of the same fragment yields knowledge of the positions of the A's and C's. The sequence is known if the procedure is repeated for the complementary strand. The system can further be used for analysis of polymer (polypeptide, RNA, carbohydrates, etc.), size, concentration, type, identity, presence, sequence and number.

Detailed Description Text (107):
In another preferred embodiment the signal generated by the interaction between the labeled unit and the agent results from fluorescence resonance energy transfer (FRET) between fluorophores. Either the labeled unit or the proximate compound/agent may be labeled with either the donor or acceptor fluorophore. FRET is the transfer of photonic energy between fluorophores. FRET has promise as a tool in characterizing molecular detail because of its ability to measure distances between two points

separated by 10 .ANG. to 100 .ANG.. The angstrom resolution of FRET has been used in many studies of molecular dynamics and biophysical phenomena (for reviews see Clegg, 1995; Clegg, 1992; Selvin, 1995; and Wu and Brand, 1994). The resolving power of FRET arises because energy transfer between donor and acceptor fluorophores is dependent on the inverse sixth power of the distance between the probes. In practice, this resolution is about an order of magnitude better than that of the highest resolution electron microscope.

Detailed Description Text (108):
In order to undergo FRET, the emission spectrum of the donor overlaps with the excitation spectrum of the acceptor. The labeled unit of the polymer is specifically labeled with an acceptor fluorophore. The agent is a donor fluorophore. A laser is tuned to the excitation wavelength of the donor fluorophore. As the polymer is moved through the channel, the donor fluorophore emits its characteristic wavelength. As the acceptor fluorophore moves into interactive proximity with the donor fluorophore, the acceptor fluorophore is excited by the energy from the donor fluorophore. The consequence of this interaction is that the emission of the donor fluorophore is quenched and that of the acceptor fluorophore is enhanced.

Detailed Description Text (109):
In order to generate an optimal efficient FRET signal for detection, two conditions should be satisfied. The first condition is efficient donor emission in the absence of acceptors. The second is efficient generation of a change in either donor or acceptor emissions during FRET. Each of these are described in more detail in co-pending PCT Patent Application PCT/US98/03024 and U.S. Ser. No. 09/134,411.

Detailed Description Text (121):
As used herein a "material shield" is any material which prevents or limits energy transfer or quenching. Such materials include but are not limited to conductive materials, high index materials, and light impermeable materials. In a preferred embodiment the material shield is a conductive material shield. As used herein a "conductive material shield" is a material which is at least conductive enough to prevent energy transfer between donor and acceptor sources.

Detailed Description Text (122):
A "conductive material" as used herein is a material which is at least conductive enough to prevent energy transfer between a donor and an acceptor.

Detailed Description Text (123):
A "nonconductive material" as used herein is a material which conducts less than that amount that would allow energy transfer between a donor and an acceptor.

Detailed Description Text (142):
In enzyme (molecular motor) or template (polymer) immobilization analysis of the complexes, a simplified reaction vessel is used which consists of high grade fused silica slide and coverslip. The enzyme or template is immobilized to the fused silica surface by different coupling means as discussed below.

Detailed Description Text (145):
Immobilization of molecular motors is accomplished using methods known in the art for immobilizing proteins. Immobilization of RNA transcription complexes, for instance, has been reported by Schafer et al, 1991. Briefly, transcription complexes were placed between two lines of silicone vacuum grease in the center of a borosilicate glass coverslip (Clay-Adams, No. 3223) in a humidified chamber and incubated for 15 minutes at 20.degree. C. To remove unbound complexes, the solution over the coverslip was partially replaced 10 times by the simultaneous withdrawal of 10 .mu.l solution and addition of 10 .mu.l PTC buffer, and BSA was then added to a concentration of 1 mg/ml. To initiate transcription, 1 mM each of ATP, CTP, GTP, UTP was allowed to flow into the chamber. Similar protocols can be followed for the immobilization of other biomolecules such as DNA polymerase and helicases. The binding of template DNA to the solid support may be accomplished by several means including streptavidin-biotin interaction or amine-succinimidyl ester linkage. These protocols are outlined beginning with the streptavidin-biotin interaction: 2.67 .mu.l of concentrated (15 pmol/L) are added to 7.33 .mu.l of water. The streptavidin coated surface, obtained from Xenopore, N.J. is placed on a sponge in a petri dish in water. 10 .mu.l of the

template mixture is added to a 1 cm.sup.2 area of slide. The petri dish is covered and incubated for one hour at room temperature. The glass is washed three times with 0.1M sodium phosphate pH 7.2 containing 0.15M NaCl. For the amine-succinimidyl ester linkage, the protocol follows. The succinimidyl ester derivitized surfaces are obtained from Corning Life Sciences, Mass. 200 .mu.L of 25 picomolar DNA in phosphate buffer is added to the surface and incubated overnight at 4.degree. C. The surface is washed three times with Tween 20 in PBS. TE buffer (10 mM Tris, pH 8, 1 mM EDTA) is added to block unreacted succinimidyl ester groups, incubating for 30 minutes at 37.degree. C. The plate is washed three times with Tween 20 in PBS.

Detailed Description Text (147):
Alternatively, the enzymes are fixed onto the surface using standard protein immobilization techniques (Schafer et al., 1991). Transcription complexes (molecular motors) are placed between two lines of silicone vacuum grease in the center of a borosilicate glass coverslip (Clay-Adams, No. 3223) in a humidified chamber and incubated for 15 minutes at 20.degree. C. To remove unbound complexes, the solution over the coverslip is partially replaced 10 times by the simultaneous withdrawal of 10 .mu.l solution and addition of 10 .mu.l PTC buffer. BSA is then added to a concentration of 1 mg/ml. The concentration of the complexes is adjusted so that by probability, there is less than one complex per grid location. The grid locations in this technique are determined by a hydrophobic/hydrophilic patterning area. Only the hydrophilic regions of the chip are derivitized with the enzyme complexes. The hydrophilic regions are arranged in a densely packed grid pattern with more than a million grid locations per square centimeter. To create the hydrophilic patterned areas, a porous/gridded silicone mask (General Electric RTV 615A and 615B) is placed over the glass area and subject to a gentle oxygen plasma etch using 25 mmTorr O2 at 25 sccm flow rate, 30 W for 50 seconds. The areas exposed to the oxygen etch are rendered hydrophilic and amenable to site specific immobilization of the enzyme-fluorophore complexes.

Detailed Description Text (166):
Single molecule data is generated from the molecular motor complex migrating along the strand of DNA, shown schematically. The data is streamed in real time to give information about the precise labeling strategy of the DNA molecule. Real-time sequence is generated from the pattern of the acceptor emissions. Excitation of the donor molecule gives rise to proximity excitation of the acceptor. Either the emission of the donor molecule is measured or the emission of the acceptor. Real-time increases in signal arises from measurement of the acceptor fluorescence and real-time decreases in signal arises from measurement of the donor fluorescence, shown in graph depicting Intensity vs Time. The raw information from single molecular analysis is directly correlated to the labeling strategy and the real-time output from the system. The information from the detectors is streamed to a databoard operating at the appropriate data rates. Software analysis in LabView or similar program yields quantitative data of DNA sequence information.

CLAIMS:

11. The method of claim 1, wherein the polymer is a nucleic acid and the molecular motor is a nucleic acid molecular motor.

12. The method of claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, wherein the molecular motor is a nucleic acid molecular motor that is a polymerase.

20. A nucleic acid molecular motor, wherein the nucleic acid molecular motor includes an agent selected from the group consisting of an electromagnetic radiation source, a quenching source, and a fluorescence excitation source positioned in interactive proximity with a signal station of the nucleic acid molecular motor.

21. A solution comprising the nucleic acid molecular motor of claim 20.

22. The solution of claim 21, wherein the nucleic acid molecular motor is a polymerase.

34. The method of claim 23, wherein the molecular motor is a nucleic acid molecular motor.

36. The method of claim 34, wherein the nucleic acid molecular motor is a polymerase.